Level of Acrylamide Precursors Asparagine, Fructose, Glucose, and Sucrose in Potatoes Sold at Retail in Italy and in the United States

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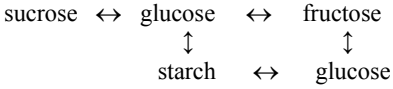
ABSTRACT: The free amino acid asparagine and the reducing sugars glucose and fructose has been reported to serve as precursors for the heat-induced formation of potentially toxic acrylamide in a variety of plant-based food. To contribute to our knowledge about the levels of these precursors, we used ion-exchange chromatography to measure free asparagine and high-performance liquid chromatography (HPLC) to measure free glucose, fructose, and sucrose in 9 potato varieties sold at retail in Italy and in 22 varieties sold in the United States. Asparagine levels (in mmol/kg of fresh wt) ranged from 1.17 for the Agata potatoes to 57.65 Russet potatoes, a 49.3-fold variation from lowest to highest value. The corresponding levels for fructose ranged from 1.73 (Fingerling Ozette) to 33.63 (Red), a 19.4-fold variation from the lowest to the highest value. For glucose, the concentration ranged from 1.11 (Jelli) to 34.73 (Yukon Gold B) potatoes, a 31.3-fold variation from lowest to highest value. The corresponding values for sucrose ranged from 1.16 (Fingerling Ozette) to 40.61 (Marabel) potatoes, a 35-fold variation. The American potato varieties Kennebec, White, and Fingerling Ozette and the Italian potato varieties Agria, Merit, and Marabel had very low levels of both asparagine and reducing sugars. The results may enable consumers, restaurants, and processors to select commercial potatoes with low levels of acrylamide precursors for baking or frying.

Keywords: analysis, acrylamide precursors, asparagine, fructose, glucose, sucrose, commercial potatoes

Introduction

Potentially toxic acrylamide in foods is largely formed by heat-induced Maillard-type reactions between the amino group of the free amino acid asparagine and the carbonyl group of reducing sugars such as glucose and fructose during baking and frying (Friedman 2003; Taeymans and others 2004). Although sucrose, a non-reducing disaccharide, does not appear to be directly involved in acrylamide formation, it can undergo enzyme-catalyzed conversion to glucose and fructose during cold-storage of potatoes at <10 °C (Isla and others 1998). In fact, free sugars and starch that exist in potato tubers are reported to participate in the so-called cold-sweetening-induced hydrolysis and epimerization (inversion) reactions subsequently illustrated schematically. See Schwimmer (1981) for a more complex elaboration of this scheme.



The indicated pathways are cultivar-dependent and reversible. They can proceed in any direction. Above 10 °C, the sugars and starch remain in balance with the free sugars either reforming into starch or being used up in other reactions. Below 10 °C, reducing sugars start to accumulate. This is undesirable because the reducing sugars may then participate in both nonenzymatic browning reactions and acrylamide formation during processing of the potatoes (Friedman 1996, 2003, 2005). Ascorbic acid browning also occurs during home-processing of potatoes (Han and others 2004).

Reconditioning potatoes at room temperature following cold storage results in significant reductions in the content of reducing sugars (Schwimmer 1984; Brierley and others 1996; Duncan and Hardie 2003). Potatoes are a major human food rich in these acrylamide precursors. However, the levels of these ingredients vary widely among potato varieties. This is because plant genetics governing their biosynthesis as well as both pre-harvest (soil composition, climate, fertilizers) and post-harvest (storage temperature and atmosphere) conditions are known to significantly influence levels of these compounds in potato tubers (Silva and Simon 2005). Processed foods with high levels of acrylamide include French fries and potato chips (Granda and others 2004).

Because asparagine and reducing sugars (fructose and glucose) are reported to be key participants in acrylamide formation, the main objective of this study was to determine the content of these acrylamide precursors in commercial potato varieties sold at retail in Italy and the United States. Since, as mentioned, sucrose and starch can be converted to glucose and fructose during storage of potatoes, we also measured the contents of sucrose in the commercial potatoes of unknown storage history. Our observations can serve as a guide to consumers and processors, who may wish to buy potatoes with low amounts of acrylamide precursors for home use.

Materials and Methods

Source of potatoes

Potatoes grown in Italy were obtained from local markets in Rome. Potatoes grown in the United States were purchased at local

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Published on Web 2/22/2006

markets in Berkeley, California. We have no other information about the history of these potatoes.

Freeze-drying of potatoes

The following method was used to freeze-dry (lyophilize) potato tubers to powders. Potatoes were rinsed in cool water and dried. They were then diced, weighed, and quickly cooled with liquid nitrogen. The samples were stored overnight in a subzero freezer. Samples were lyophilized over a period of 5 d, after which they were then removed from the lyophilizer, weighed, and stored in a zip-lock plastic bag until ready to grind. They were ground batch-wise in a Waring blender (Waring Laboratory, Torrington, Conn., USA) and sieved through a 0.5-mm screen. Pieces, which would not pass through the screen, were ground in a mortar and pestle and passed through the sieve. Samples were then double-bagged and stored in the freezer. The freeze-dried powders from potatoes grown in the United States (American potatoes) were then sent to Rome, Italy, for analysis.

Analysis of asparagine

The automated ninhydrin assay (Friedman and others 1984; Friedman 2004) was adapted from the literature for free amino acids including asparagine (Mondino 1969; Mondino and others 1972). Briefly, the lyophilized (freeze-dried) (approximately 100 mg) sample was deproteinized by stirring in 50 mL of a 0.3 *M* sulfosalicylic acid solution for 5 min. This was followed by centrifugation at 8200 × *g* for 5 min. An aliquot of the supernatant (250 μL) was injected into a Beckman 118 BL amino acid analyzer (Beckman Coulter, Inc., Fullerton, Calif., USA). Cysteic acid was used as the internal standard.

Analysis of fructose, glucose, and sucrose

The procedure was adapted with modifications from Varian (2004). Potato samples (1 g) of fresh Italian or of freeze-dried (200 mg) American potatoes in 10 mL acetonitrile/water (80:20 v/v) were stirred for 5 min. The suspension was then centrifuged at 1700 × *g* for 10 min and the supernatant was passed through a 0.45-μm Millex filter (Millipore, Bedford, Mass., U.S.A.). Aliquots (50 μL) of the filtrate were injected into a Beckman 342 high-performance liquid chromatography (HPLC) instrument equipped with a refractive index (R.I.) detector (Palo Alto, Calif., U.S.A.) and an Inertsil NH_2, 4 × 250-mm column (GL Sciences, Torrence, Calif., USA). An isocratic mode of elution was used with the mobile phase consisting of acetonitrile/water (80:20, v/v) at a flow rate of 0.5 mL/min. Maltose was used as an internal standard.

Quantification and identification

Each peak was identified by comparing the retention times and peak areas of unknowns with those of standards. Identification was confirmed by spiking sample peaks with known standards. Potato extracts were analyzed before and after addition of known amounts of standard compound. Recovery (%) = (concentration of each compound in spiked sample)/concentration of endogenous compound + spike) × 100.

Results and Discussion

Analytical aspects

Asparagine. After extraction from the plant matrix, analysis for free asparagine and other free amino acids is a challenging problem because they often co-elute with other protein and non-protein amino acids present in the extracts. To overcome this problem, Vasinitis and Molnar-Perl (Vasanits and Molnar-Perl 1999) developed protocols for the analysis for free amino acids in apples by HPLC. Jia and others (Jia and others 2001) describe a 2-step ion-chromatographic procedure for the analysis of asparagine. The 1st analysis yields data for free aspartic acid, and the 2nd step involves hydrolysis of asparagine to aspartic acid followed by analysis for the asparagine-derived aspartic acid. Martin and Ames (Martin and Ames 2001) used capillary electrophoresis to measure the content of asparagine in fresh and fried potato slices. Rozan and others (Rozan and others 2001) analyzed asparagine and other free amino acids in lentils.

To further improve determination of free asparagine in plant materials, we adapted the method from the literature for free amino acids in biological samples (Mondino 1969; Mondino and others 1972) to the analysis of asparagine in potato extracts. The results in Figure 1 show that asparagine is well separated from aspartic acid on an ion-exchange column of an amino acid analyzer, eluting between serine and glutamic acid. Results from 2 separate spiking experiments of a potato sample showed a 96.0% and 96.9% recoveries of asparagine. A single-step ion-exchange method for asparagine in potato extracts we developed for the present study complements previously reported procedures mentioned earlier. The method merits application to free asparagine in other plant foods.

Fructose, glucose, and sucrose. We found that the Inertsil HPLC column, which we previously used for the analysis of potato glycoalkaloids (Friedman and others 2003), was also found to be well-suited for the separation of free sugars present in potato extracts, as indicated by the complete separation of HPLC peaks associated with a mixture of authentic fructose, glucose, and sucrose, and the internal standard maltose (not shown). Two detection methods were used for quantitation of the results: refractive index (nRIU) and ultraviolet spectroscopy (mAU). Examination of the standard curves (not

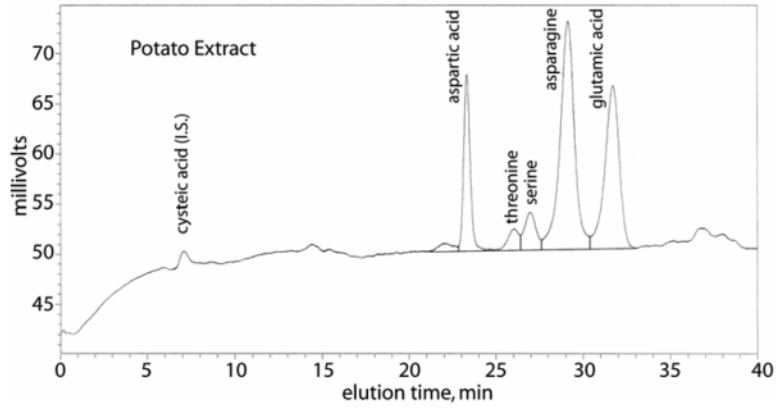

Figure 1—Ion-exchange chromatogram of a potato extract plus cysteic acid used as the internal standard (I. S.). Note that asparagine elutes between serine and glutamic acid, well separated from the aspartic acid peak.

shown) indicates linearity in the region from 0.05 to 0.20 mg/mL for the 4 sugars. Results from 2 separate spiking experiments of a potato sample resulted in a 93.1% and 95.5% recoveries of maltose.

Levels of asparagine and free sugars in commercial potatoes

Asparagine. Table 1 lists the composition data for asparagine, fructose, glucose, sucrose, and water for 9 Italian potato varieties and Table 2 lists the corresponding values for the 22 American potato samples. The data show that the asparagine levels of the 9 Italian potato varieties ranged (in mmol/kg of fresh wt) from 1.17 (Agata) to 34.70 (Spunta), a 29.7-fold variation from lowest to highest value. The asparagine levels of the American potato varieties ranged (in mmol/kg of fresh wt) from 3.76 (Purple, Peruvian) to 57.65 (Russet 2), a 15.6-fold variation from lowest to highest value.

The following 3 Italian varieties contained <10 mmol of asparagine/kg of fresh potato wt: Spunta, 1.67; Jelli, 3.89; and Agria, 6.67. The following 3 American varieties also contained < 10 mmol/kg of fresh wt: Kennebec, 3.15; White, large, 6.41; Fingerling Ozette 1, 8.64.

Fructose, glucose, and sucrose, and water. Table 1 shows that for the 9 Italian potatoes, the concentration of fructose (in mmol/kg of fresh wt) ranged from 0 (Spunta) to 9.99 (Finka). For glucose, the corresponding range was from 1.11 (Jelli) to 18.87 (Arinda), a 17-fold variation. For sucrose, the corresponding range was from 9.64 (Jelli) to 40.61 (Marabel), a 4.2-fold variation. The water content of the 9 varieties (in %) ranged from 75.20 (Spunta) to 81.90 (Arinda and Marabel).

The following 3 Italian varieties had the lowest amount of glucose plus fructose (in mmol/kg of fresh wt): Spunta, 1.67; Jelli, 3.89; and Agria, 6.67. The sums of the 2 reducing sugars in the other 6 varieties ranged from 12.77 (Marabel) to 23.31 (Arinda), with 3 of the 6 at >20 mmol/kg.

For the 22 potato tubers originating in the United States, the concentration of fructose (in mmol/kg of fresh wt) ranged from 1.06 (White, medium) to 33.36 (Red, large), a 31.5-fold variation from lowest to highest value. For glucose, the corresponding range was from 0.85 (Kennebec) to 34.73 (Yukon Gold, grade "B," medium), a 40.6-fold variation from lowest to highest value. For sucrose, the corresponding range was from 1.16 (Fingerling Ozette 1) to 10.40 (White, medium), a 9-fold variation. The water content (in %) of all potato cultivars ranged from 75.02 (Kennebec) to 84.25 (Red Creamer, marble).

Lack of a relationship between asparagine and sugar levels

There does not seem to be any apparent relationship between increasing levels asparagine in different potatoes and the corresponding sugar levels. This is clearly evident from an examination of Figure 2, which compares the levels as bar graphs in increasing order of asparagine to the sum of the reducing sugars (glucose plus sucrose) of 9 Italian and 22 American potato samples.

Table 1—Composition of Italian potatoes listed in order of increasing levels of asparagine

	mmol/kg of fresh weight (n = 3)				
Potato variety	Asparagine	Fructose	Glucose	Sucrose	Water (%)
Agata	1.17 ± 0.07	9.44 ± 0	12.77 ± 0.56	15.48 ± 0.29	80.60 ± 0.21
Arinda	3.81 ± 0.18	4.44 ± 0	18.87 ± 1.67	16.65 ± 1.46	81.90 ± 0.12
Agria	11.10 ± 0	2.78 ± 1.11	3.89 ± 1.67	10.52 ± 0.58	80.10 ± 2.06
Merit	11.68 ± 0.47	7.22 ± 0	9.99 ± 0	22.79 ± 0.88	79.00 ± 3.26
Marabel	13.85 ± 0.70	6.11 ± 0.56	6.66 ± 0	40.61 ± 4.09	81.90 ± 0.40
Jelli	18.03 ± 0	2.78 ± 0	1.11 ± 0	9.64 ± 0.58	78.90 ± 1.50
Finka	22.29 ± 0	9.99 ± 0	12.21 ± 5.55	13.44 ± 1.17	78.90 ± 2.15
Primura	29.69 ± 1.29	7.22 ± 0	11.66 ± 1.11	10.22 ± 0.29	80.90 ± 0.06
Spunta	34.70 ± 0	0 ± 0	1.67 ± 0.56	13.15 ± 0.58	75.20 ± 1.12

Table 2—Composition of American potatoes listed in order of increasing levels of asparagine

	mmol/kg of fresh weight (n = 3)				
Potato variety	Asparagine	Fructose	Glucose	Sucrose	Water (%)
Purple, Peruvian	3.76 ± 0.26	24.03±0.27	17.14±0.19	7.88 ±0.18	77.27
Red, medium, organic	5.86 ± 0.40	11.22±0.32	15.67±0.33	4.30 ± 0.08	82.33
Yukon Gold, grade "B," medium	6.13 ± 0.39	28.42±0.06	34.73±1.68	7.65 ± 0.27	75.49
Red, grade "A," large	6.33 ± 0.48	21.21±0.49	24.63±1.33	5.13 ± 0.10	80.87
Ruby Red Crescent	6.49 ± 0.55	16.26±0.23	26.08±0.09	8.11 ± 0.47	78.89
Fingerling Ozette 1[a]	6.80 ± 0.51	8.06 ± 0.32	6.43 ± 0.29	1.16 ± 0.12	79.97
Yukon Gold, grade "C," small	7.04 ± 0.54	16.38 ± 0.08	18.95 ± 1.64	3.71 ± 0.34	82.31
Yukon Gold, grade "A," large	8.05 ± 0.63	12.62 ± 0.10	15.26 ± 1.01	3.63 ± 0.31	76.89
White, medium	8.18 ± 0.73	1.06 ± 0.15	26.97 ± 0.48	10.40 ± 0.06	79.36
Kennebec	8.76 ± 0.79	2.30 ± 0.27	0.85 ± 0.08	1.46 ± 0.08	75.02
Purple, large	8.97 ± 0.67	12.01 ± 0.44	13.78 ± 0.32	9.06 ± 0.09	75.25
Yukon	9.75 ± 0.41	23.88 ± 0.21	34.55 ± 0.29	6.23 ± 0.11	81.72±0.16
White, large	10.67 ± 0.85	2.04 ± 0.17	4.37 ± 0.21	2.36 ± 0.27	79.93
Red, grade "C," small	11.02 ± 0.76	14.76 ± 0.28	19.12 ± 0.26	3.89 ± 0.33	81.30
White Creamer, small	11.40 ± 1.07	10.46 ± 0.53	19.28 ± 1.03	3.26 ± 0.13	83.41
Butterball Creamer, organic, German	19.60 ± 1.70	9.58 ± 0.17	12.75 ± 0.06	3.53 ± 0.19	80.47
Red, large	19.67±0.92	33.36 ± 0.22	33.67 ± 0.32	4.31 ± 0.15	79.96±0.72
Red Creamer, marble	20.36 ± 1.59	11.01 ± 0.12	21.09 ± 0.41	3.17 ± 0.23	84.25
Fingerling French	20.77 ± 1.74	16.96 ± 0.18	16.29 ± 0.46	8.78 ± 0.16	79.27
Russet 1[a]	25.35 ± 2.15	8.11 ± 0.13	7.72 ± 0.49	4.03 ± 0.16	79.49
Fingerling Ozette 2[a]	28.70 ± 2.48	1.73 ± 0.07	6.91 ± 0.02	1.52 ± 0.04	78.51
Russet 2[a]	57.65 ±2.71	11.34 ± 0.13	16.94 ± 0.18	4.38 ± 0.39	80.18±0.93

[a]Fingerling Ozette 1 and Fingerling Ozette 2 potatoes were obtained from different markets, as were Russet 1 and Russet 2 potatoes.

For the combined 31 American and Italian potato samples, the data in Tables 1 and 2 and Figure 2 show that the range for asparagine concentrations (in mmol/kg of fresh wt) was from 1.17 (Agata) to 57.65 (Russet 2), a 49.2-fold variation from highest to lowest value. For fructose, the corresponding range was from 1.73 (Fingerling Ozette 2) to 33.63 (Red, large), a 19.4-fold variation from highest to lowest value. The corresponding range for glucose was from 1.11 (Jelli) to 34.73 (Yukon Gold, grade "B," medium), a 31.3-fold variation from lowest to highest value. The corresponding range from sucrose was from 1.16 (Fingerling Ozette 1) to 40.61 (Marabel), a 35-fold variation from lowest to highest value. The lack of parallelism between free asparagine and free sugar content implies that the biosynthesis of these potato components in the plant is under different genetic control (Duncan and Hardie 2003; Silva and others 2005).

Significance for acrylamide formation

What do the cited data mean for the consumer of potatoes? Our results demonstrate large variations in free asparagine as well as free fructose, glucose, and sucrose content of both Italian and American potatoes. To place these results in proper perspective, it is instructive to examine the following reported relationships of levels asparagine and reducing sugars to the formation of acrylamide in potato matrices formed under the influence of various processing conditions.

Amrein and others (Amrein and others 2003) found that acrylamide formation in 74 different potato cultivars correlated with their content of fructose and glucose. Asparagine had less of an effect, presumably because, in contrast to reducing sugars, its content among the cultivars did not vary widely.

Chuda and others (Chuda and others 2003) found that acrylamide levels were highly correlated with both glucose and fructose levels of potato tubers. In a related study on the influence of free amino acids and sugars on acrylamide formation, Becalski and others (2004) found that acrylamide levels of French fries can be minimized by using potatoes with low levels of sugars (fructose, glucose, and sucrose) and to a lesser extent low levels of asparagine. Williams (Williams 2005) also found that acrylamide levels in 5 potato varieties were significantly influenced by reducing sugars rather than by asparagine. In contrast, Rydberg and others (Rydberg and others 2005) reported that both asparagine and reducing sugars increased levels of acrylamide in heated potato slurries.

Olsson and others (Olsson and others 2004) found that asparagine levels increased during storage of potatoes. They suggest that the choice of potato clones with low asparagine content might minimize risk that the amino acid will increase during long-term storage. In contrast, Yoshida and others (Yoshida and others 2005) found that asparagine content of the Japanese Toyoshiro potato tubers did not change during cold storage. In a related study on the effects of storage, Silva and Simon (Silva and Simon 2005) found that glucose, fructose, sucrose, and asparagine levels in potato tubers increased during storage at 2 °C and that glucose and fructose (but not sucrose and asparagine) levels correlated with acrylamide formation. They suggested that because tuber sugar and asparagine levels may be affected by cultural practices, genetics, and storage, breeding of potatoes for low asparagine levels might result in reduction in acrylamide content of processed potato products.

Studies by Jackson and Al-Taher (Jackson and Al-Taher 2005) revealed that cooling potatoes to temperatures <10 °C caused an increase in reducing sugars and a consequent increase in acrylamide levels of fried potatoes, suggesting that the sugars appear to determine the extent of acrylamide formation.

Recently, De Wilde and others (2005) found that acrylamide levels in fried potatoes derived from different potato varieties correlated with the reducing sugar content of the potatoes ($R^2 = 0.84$; $n = 160$)

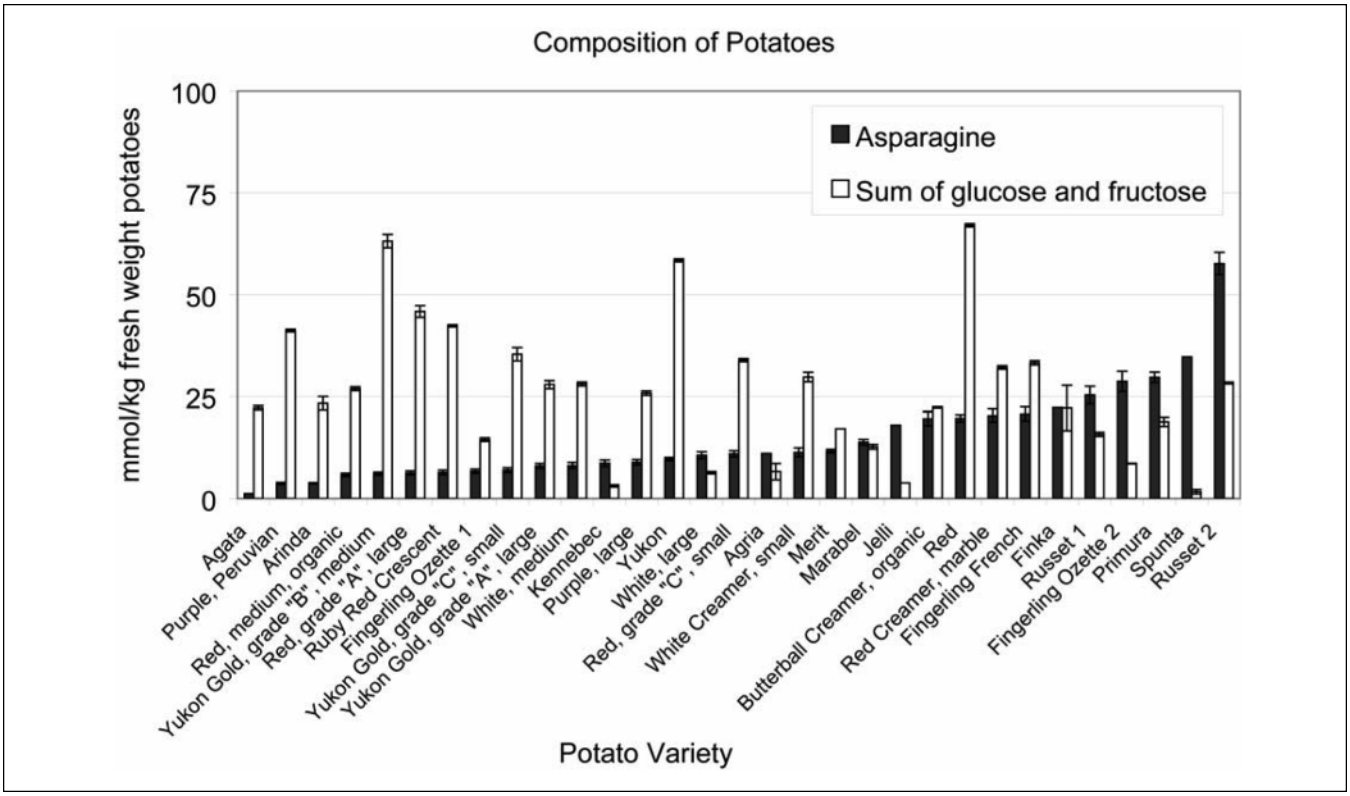

Figure 2—Relationship between increasing levels of asparagine in 31 potato samples and the sum of glucose and fructose. Some of the standard deviations are low and are difficult to see on the bars.

and that reconditioning of cold-stored potatoes for 3 wk at 15 °C resulted in significant reduction in the levels of reducing sugars.

On balance, the cited studies imply that our data on reducing sugar content of "retail" potatoes may predict expected relative acrylamide levels of the different varieties after processing.

Significance for consumers and research needs

Our results and the cited observations by other investigators suggest that to minimize acrylamide content of the diet, the consumer could select from the commercially available potato varieties those which are low in both asparagine and reducing sugar content. For example, our data show that for the American varieties Kennebec, White, and Fingerling Ozette potatoes had very low amounts of both asparagine and reducing sugars. The Italian potatoes Agria, Merit, and Marabel varieties also had very low levels of both acrylamide precursors. In this regard, it is relevant to note that to reduce exposure of consumers to acrylamide, Swiss authorities recommended that potatoes low in reducing sugars to be used for frying and roasting should be sold separately from potatoes for other uses (Grob 2005).

It is, however, also relevant that both asparagine and sugar levels may vary for the same potato variety purchased in different stores and at different times, as indicated by the data in Table 2 for Fingerling Ozette 1, Fingerling Ozette 2, and Fingerling French; Red, medium, organic, Red Creamer, marble, Red, grade "A," large, and Red, grade "C," small; and Russet 1 and Russet 2 potatoes. Table 2 also shows that the 3 Yukon Gold potatoes (Yukon Gold, grade "A," large; Yukon Gold, grade "B," medium; Yukon Gold, grade "C," small) did not significantly differ in their content of asparagine. As mentioned earlier, reconditioning potatoes after cold storage usually results in decreases in the levels of free sugars, a desirable goal for minimizing processing-induced acrylamide formation.

Conclusions

The cited studies together with our observations suggest that (a) a measurement of acrylamide precursors, especially reducing sugars, of potato varieties at a specific time may not always reflect levels present at earlier or later time periods; (b) to reduce cold-storage-induced sugar levels, potatoes should be reconditioned after storage; (c) post-harvest handling of potatoes should be standardized to minimize formation of reducing sugars; (d) plant scientists are challenged to develop potato cultivars that resist forming additional reducing sugars during cold storage; (e) ideally, to serve as a guide, the content of reducing sugars should be determined immediately before home and commercial processing of the potatoes; (f) labeling potatoes sold at retail for reducing sugar content may benefit consumers (Grob 2005); and (g) such labeling may be facilitated by use of an inexpensive instruments that can rapidly measure glucose and fructose levels of whole potato tubers, analogous to the use of such instruments to measure plasma sugar levels of diabetics (Schaepellynck-Belicar and others 2003).

Acknowledgments

We are most grateful to Carol E. Levin for freeze-drying potato tubers.

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